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# Lactose-over-Glucose Preference in *Bifidobacterium longum* NCC2705: *glcP*, Encoding a Glucose Transporter, Is Subject to Lactose Repression†

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**Analysis of culture supernatants obtained from *Bifidobacterium longum* NCC2705 grown on glucose and lactose revealed that glucose utilization is impaired until depletion of lactose. Thus, unlike many other bacteria, *B. longum* preferentially uses lactose rather than glucose as the primary carbon source. Glucose uptake experiments with *B. longum* cells showed that glucose transport was repressed in the presence of lactose. A comparative analysis of global gene expression profiling using DNA arrays led to the identification of only one gene repressed by lactose, the putative glucose transporter gene *glcP*. The functionality of GlcP as glucose transporter was demonstrated by heterologous complementation of a glucose transport-deficient *Escherichia coli* strain. Additionally, GlcP exhibited the highest substrate specificity for glucose. Primer extension and real-time PCR analyses confirmed that expression of *glcP* was mediated by lactose. Hence, our data demonstrate that the presence of lactose in culture medium leads to the repression of glucose transport and transcriptional down-regulation of the glucose transporter gene *glcP*. This may reflect the highly adapted life-style of *B. longum* in the gastrointestinal tract of mammals.**

Bifidobacteria are strictly anaerobic microorganisms that are found as commensals in the mammalian gastrointestinal tract (2). They predominate in infants' intestines and can represent up to 3% of the gut microbiota in adult humans (2). Together with lactobacilli, bifidobacteria are considered health-promoting bacteria and thus are used as food additives in the dairy industry (1, 9).

Bifidobacteria can utilize a wide range of carbon sources. Some of them, such as oligofructose, inulin, and raffinose, have been identified as growth-promoting, bifidogenic compounds (8, 10). This is further substantiated by sequence information from *Bifidobacterium longum* NCC2705, whose chromosome encodes a large variety of carbohydrate utilization genes (22). Nevertheless, little is known about the mechanisms of simple sugar transport, utilization, and regulation in bifidobacteria. Biochemical analyses of glucose transport revealed that a glucose-specific phosphotransferase system (PTS) is present in *Bifidobacterium breve*, and a potassium-dependent glucose permease, a facilitator for galactose, and a proton-driven symporter for lactose were described in *Bifidobacterium bifidum* (5, 12, 13). A sucrose permease gene from *Bifidobacterium lactis* was found as part of an operon that is induced by sucrose and raffinose and is subject to glucose repression (23). The isolation of a fructose kinase gene, *frk*, which is also repressed by glucose, has been related to fructose utilization in *B. longum* (3).

In this report, we demonstrate that *B. longum* NCC2705 preferentially uses lactose over glucose when grown in the presence of both sugars. We further show that glucose transport is down-regulated by lactose, and we identify a glucose transporter gene that undergoes lactose repression.

## MATERIALS AND METHODS

**Bacterial strains and culture conditions.** *Bifidobacterium longum* NCC2705 was isolated from human infant feces (22). Cells of *B. longum* were grown anaerobically at 37°C either as a static culture using the gas pack system (AnaeroGen; Oxoid) or with stirring (200 rpm) in a 0.5-liter fermenter (Sixfors; INFORS) under a $CO_2$ atmosphere. Fresh de Man Rogosa Sharpe medium (MRS; Difco) or semisynthetic growth medium (SSM) (19) supplemented with 5 mM L-cysteine were used. *Escherichia coli* DH5α served as a host strain for subcloning experiments, and the glucose transport-deficient *E. coli* mutant LM1 [*tonA galT nagE manA1 kba*(Ts) *rplI xyl metB thi his mglA agrG crr*] (15) was used for heterologous complementation experiments. *E. coli* strains were grown in Luria-Bertani broth supplemented with ampicillin (100 mg liter$^{-1}$), where appropriate, in baffled flasks under vigorous shaking at 37°C. Bacterial growth was monitored by measuring the optical density at 600 nm ($OD_{600}$).

***glcP* cloning strategy.** Chromosomal DNA of *B. longum* was isolated following the procedure of Germond et al. (7). Two DNA fragments of 2,031 bp and 1,605 bp comprising *glcP* (accession number AAN25419) of *B. longum*, with and without its upstream region, were amplified from chromosomal DNA by PCR with Taq-Hifi DNA polymerase (Roche, Germany) using pairs of oligonucleotides glcP1 (AATCAGGATCCGTGTGCCGGTTGTCTAGC; BamHI site underlined) and glcP3 (TATCGAAGCTTGGTGCCCCAAAAGCACGGC; HindIII site underlined) and glcP1 and glcP2 (GCAACAAGCTTGGTCCGTTAACGGCGAAAGG; HindIII site underlined), respectively. glcP3 and glcP2 were designed to provide a stop codon in frame with the *lacZ* gene encoded by pBluescript II KS(−). The PCR fragments were cloned into BamHI and HindIII sites of pBluescript II KS(−) to produce plasmids pMB1 and pMB2, harboring *glcP* without and with its upstream region, respectively.

**Glucose transport assays.** For glucose transport experiments *B. longum* was grown in Sixfors fermenters on SSM supplemented with 10 mM glucose, 5 mM lactose, and 10 mM glucose plus 5 mM lactose. A culture (50 ml) of exponentially growing cells was taken at $OD_{600}$ of 0.5. Cells were immediately harvested at room temperature and washed twice in SSM, saturated with $CO_2$ and 5 mM L-cysteine, at 37°C. Cells were adjusted to $OD_{600}$ of 1.0 and kept statically at 37°C under anaerobic conditions using the gas pack system. Samples were sequentially taken from the gas pack system, and glucose uptake was initiated by addition of [$^{14}$C]glucose at a final concentration of 200 μM (0.25 mCi mmol$^{-1}$) without

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delay. Glucose transport activity was determined by taking 1-ml samples after 60 s of incubation at 37°C, which was within the linear range of transport. Samples were rapidly filtered through nitrocellulose filter disks (NC45; Schleicher & Schuell) and washed with chilled 0.1 M LiCl. Radioactivity was determined by scintillation oscillography.

The determination of $K_m$ and $V_{max}$ of *B. longum* GlcP was carried out in *E. coli* LM1(pMB1). The heterologous expression of *glcP* was induced in *E. coli* for one hour at early exponential growth phase ($OD_{600}$ of 0.5) with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). Cells were harvested and washed twice with chilled transport buffer (Tris-HCl, pH 7.5, 50 mM; NaCl, 50 mM; KCl, 10 mM), adjusted to $OD_{600}$ of 1 and kept on ice. Glucose uptake was conducted at 37°C as described above.

Substrate competition experiments were performed with *E. coli* LM1(pMB1). The heterologous expression of *glcP* was induced in *E. coli* for one hour at early exponential growth phase ($OD_{600}$ of 0.5) with cells to which an excess of 10 mM nonradioactive carbon source was added 1 min prior to addition of [$^{14}$C]glucose at a final concentration of 200 μM (0.25 mCi mmol$^{-1}$). The initial uptake rates were determined at final glucose concentrations in the range of 0.8 to 500 μM. All data were collected in triplicate and reproduced in three independent experiments.

**NMR experiments.** *B. longum* was grown on SSM supplemented with 10 mM [$^{13}$C]glucose and 5 mM [$^{12}$C]lactose in fermenter. Samples (1.5 ml) of growing culture were taken at different time points. Filtered culture supernatants were subjected to nuclear magnetic resonance (NMR) analysis. Samples were prepared by adding 40 μl deuterium oxide as the magnetic field lock substance and 10 μl of a 50 mM solution of TSP [(3-trimethylsilyl)-D4-propionic acid sodium salt] in $D_2O$ to 450 μl of filtered cell extracts. The samples were transferred to 5-mm-diameter NMR tubes and measured at 300 K. $^1$H NMR spectra were acquired on a Bruker DRX600 NMR spectrometer operating at a proton NMR frequency of 600.13 MHz. A standard one-dimensional pulse sequence (RD-90°-t1-90°-tm-90°-acquisition) with water presaturation during the relaxation delay (RD) and the mixing time (tm) was applied. Typical acquisition parameters included 32,000 data points, 64 transients, a spectral width of 8,389 Hz, a mixing time of 100 ms, an acquisition time of 1.95 s, and a relaxation delay of 2 s. As for standard one-dimensional NMR spectra, an exponential line-broadening function of 0.3 Hz was applied to the free induction decay prior to Fourier transformation. All spectra were referenced to TSP at 0.00 ppm and were phase and baseline corrected. For quantitative analysis, peak heights at 5.374 ppm assigned to $^{13}$CH-α1-glucose and at 4.45 ppm assigned to CH-1′-lactose were measured and referenced to an internal standard at 7.728 ppm. The detection limit of the method is 50 μM.

**Determination of sugar concentrations by HPLC.** *B. longum* was grown in fermenter containing SSM supplemented with 10 mM glucose, 5 mM lactose, or in a combination of both sugars. Culture samples (1.5 ml) were taken at different time points. Sugar concentrations were determined from filtered supernatants by high-performance liquid chromatography (HPLC) (series 1100 system; Hewlett Packard, Waldbronn, Germany). Samples (20 μl) were injected on an Aminex HPX-87H column (Bio-Rad) connected to a cation-H guard column (Bio-Rad) at 35°C. Sugars were eluted with 5 mM sulfuric acid at a flow rate of 0.6 ml/min for 25 min. The compounds were detected with a refractometer heated at 35°C and were compared to standards.

**RNA preparation.** *B. longum* was grown in fermenter containing SSM supplemented with 100 mM glucose, 50 mM lactose, or 50 mM glucose and 25 mM lactose. Culture samples (15 ml) were taken during exponential growth phase at $OD_{600}$ of 0.5. Cells were harvested, immediately frozen with liquid nitrogen, and stored at −80°C. Isolation of total RNA from *B. longum* was performed following the method of Kuipers et al. (14) with the following modifications: macaloid/phenol/sodium dodecyl sulfate/bacteria suspensions were homogenized in a bead beater (Mini-Bead Beater-8; BioSpec) for 3 × 1 min and centrifuged to remove glass beads and cell debris. After phenol extraction, total RNA was precipitated from aqueous phase with ethanol, resuspended in 100 μl diethylpyrocarbonate-treated water, and stored at −80°C. DNase treatment of total RNA was performed with an RNeasy kit (QIAGEN). RNA quality was controlled using an Agilent 2100 bioanalyzer by looking at the integrity of 16S and 23S rRNAs.

**Microarray experiments.** Glass microarrays were produced by Eurogentec SA (Belgium) by printing PCR-based probes designed to cover approximately 97% of the identified open reading frames of the *B. longum* NCC2705 genome. Labeling of RNA, synthesis of cDNAs, and hybridizations were performed using a 3DNA Array 350RP Genisphere kit (Genisphere Inc., Hatfield, Pa.), following the protocol provided by the supplier. Relative gene expression ratios were then calculated using expression of glucose-grown cells as a reference. Mean results of each comparison represent the average of four hybridizations from two biological repeats (two hybridizations for each biological repeat). Genes were considered differentially expressed if they displayed an average absolute $\log_2$-tranformed gene expression ratio of ≥1 and a $P$ value of ≤0.002. Detailed description of the method is provided as supplementary material.

TABLE 1. Primers used for real-time PCR

| Gene | Accession number | Primer pair |
|---|---|---|
| BL1631 (*glcP*) | AAN25419 | BL1631-F1, GCAACGCCTTCAAAGTGCTT |
| | | BL1631-R1, TGCACCTGGGTCTGATCCA |
| BL1630 (*pgm*) | AAN25418 | BL1630-F1, TCAAGCGTGTGCCCTACGA |
| | | BL1630-R1, GTGCTCGCGGAAGTCGAA |
| BL1632 (*ptsG*) | AAN25420 | BL1632-F1, CACCGACGCCGAACAAA |
| | | BL1632-R1, GCGTGAAGTGACGGGATGTA |
| BL1633 (*licT*) | AAN25421 | BL1633-F1, CTGACCATCTGGCGCAGAT |
| | | BL1633-R1, ACCACGCGGATGATTTCG |
| BL0274 | AAN24115 | BL0274-F1, CCGGCATGTTCCCGATT |
| | | BL0274-R1, TCAATGAATGCGGTTTCGTAAG |
| BL0301 | AAN24141 | BL0301-F1, CAACCGCCGCGATCTTC |
| | | BL0301-R1, CCAGCTGTGAAAGCAACGTATT |

**Real-time PCR.** cDNAs were synthesized from RNAs using random hexamers and TaqMan reverse transcription reagents (Applied Biosystems, Roche) according to the supplied protocol. Real-time PCR was performed in an ABI PRISM 7000 machine (Applied Biosystems) using the SYBR Green PCR Master Mix (Applied Biosystems) following the supplied protocol. Average relative gene expression values were calculated by combining data from three independent biological replicates. The normalization of real-time PCR results was done using two genes: BL0274, encoding L-ribulokinase (EC 2.7.1.16), and BL0301, encoding a cysteinyl-tRNA synthetase (EC 6.1.1.16), whose expression was shown to be constitutive under different experimental conditions (data not shown). Primers designed with the PRIMEREXPRESS software (Applied Biosystems) are presented in Table 1.

**Primer extension analysis.** Primer extension analyses were performed using AMV reverse transcriptase (Sigma-Aldrich) with 20 μg of total RNA and primer glcP3-IR (GCTCCAGGTCTTGCCTCG), which was labeled with an IRD800 infrared dye at the 5′ end. In parallel, DNA sequencing reactions were performed on the pMB2 plasmid according to the chain-terminating sequencing method using a fluorescent-labeled primer cycle sequencing kit (Amersham, Germany) and the same primer as above. Reverse transcripts together with DNA sequencing reactions were run on 8% polyacrylamide-urea gels and detected in a Li-Cor DNA sequencer.

**Computer analysis.** Protein databank searches were carried out at the BLAST server of the National Center for Biotechnology Information at the National Institutes of Health, Bethesda, Md. (http://www.ncbi.nlm.nih.gov), and the BLAST server of the Transport Classification Databank, TCDB (www.tcdb.org).

## RESULTS

***Bifidobacterium longum* preferentially uses lactose over glucose.** To analyze the preference of the sugar utilization of *B. longum* NCC2705, the strain was grown in fermenter on SSM supplemented with 10 mM glucose and 5 mM lactose. A time course of lactose and glucose depletion from the culture medium was monitored by HPLC (Fig. 1). Lactose was the first sugar disappearing from the medium. Glucose concentrations slightly decreased over time, which contrasts the rapid decline of glucose when grown in the absence of lactose (data not shown). To exclude the possible accumulation of glucose moiety from lactose in the culture media, *B. longum* was grown in the presence of 10 mM [$^{13}$C]glucose and 5 mM nonlabeled [$^{12}$C]lactose. NMR analysis showed no accumulation of [$^{12}$C]glucose or [$^{12}$C]galactose in the supernatant, indicating that both sugar moieties of lactose were metabolized. The profiles of lactose and glucose utilization obtained by HPLC and NMR were identical. Taken together, the data demonstrate that *B. longum* prefers the more complex lactose over glucose as a primary carbon and energy source.

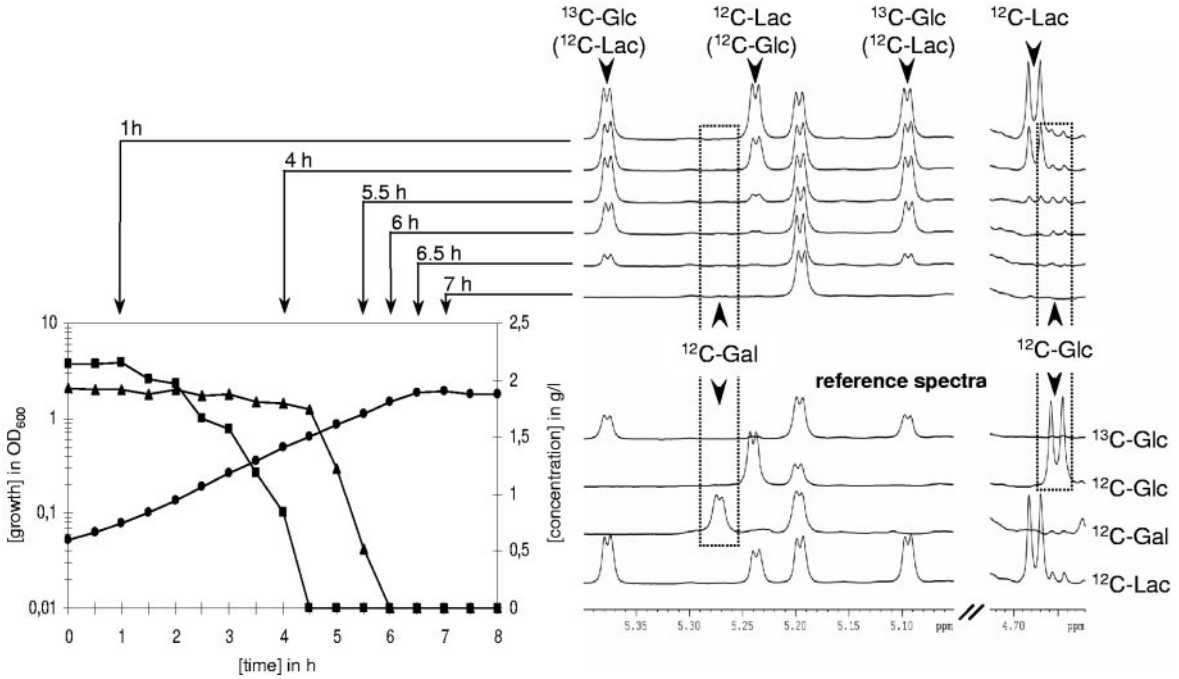


FIG. 1. Sugar depletion from culture medium containing lactose (■) and glucose (▲) during growth of *B. longum* (●). The strain was grown on SSM supplemented with 10 mM glucose and 5 mM lactose. Changes in cell density were recorded every hour. Sugar analysis was done by HPLC. For NMR analysis, the strain was grown on SSM supplemented with 10 mM [$^{13}$C]glucose and 5 mM [$^{12}$C]lactose. The NMR spectra on the right correspond to the most relevant time points showing lactose and glucose depletion. The missing peaks of [$^{12}$C]glucose and [$^{12}$C]galactose moieties of lactose are framed. Reference spectra below represent 10 mM glucose (Glc) or galactose (Gal) and 5 mM lactose (Lac) in nonfermented SSM.

**Lactose represses glucose uptake.** Glucose uptake experiments with *B. longum* cells were performed to answer the question of whether the glucose transport step is repressed by lactose. Transport measurements with the oxygen-sensitive *B. longum* were only possible when cells were rapidly treated as outlined in Material and Methods. When *B. longum* was grown on glucose, overall transport activity was 6.10 (±0.44) nmol glucose $OD_{600}^{-1}$ $min^{-1}$ (100%) (Fig. 2). When grown on glucose and lactose, cells retained 38% of the glucose transport activity, while in the presence of only lactose, the rate of glucose uptake dropped to 30%. This result shows that glucose uptake in *B. longum* cells is repressed by lactose and probably induced by glucose.

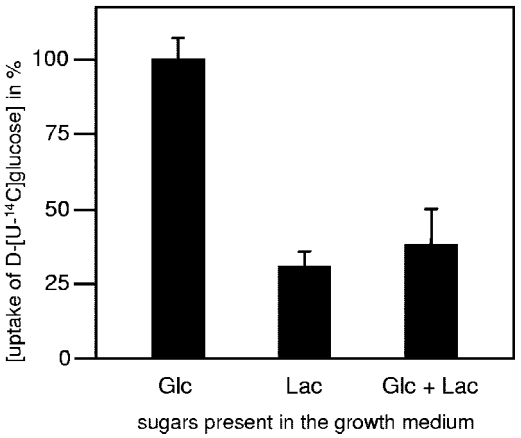


FIG. 2. Glucose uptake by *B. longum* cells. The cells were grown on SSM supplemented with 10 mM glucose (Glc), 5 mM lactose (Lac), and 10 mM glucose plus 5 mM lactose (Glc+Lac) to $OD_{600}$ of 0.5. Glucose uptake was determined using 200 μM [$^{14}$C]glucose. Glucose uptake activities are presented as a percentage of the uptake by cells grown on glucose (100% = 6.10 [±0.44] nmol of glucose $OD_{600}^{-1}$ $min^{-1}$).

**BL1631 expression is mediated by lactose.** Comparative analysis of gene expression using genome-wide DNA arrays of *B. longum* led to the identification of one gene, BL1631, which was repressed in cells grown on lactose (2.19-fold) or lactose and glucose (2.06-fold) compared to cells grown on glucose (data not shown).

Quantitative real-time PCR using the same RNA samples was performed to validate the microarray results. In this experiment, BL1631 expression was on average 2.6-fold lower in cells grown on lactose compared to cells grown on glucose (95% confidence interval between 1.6 and 4.0). In the presence of glucose and lactose on the other hand, expression of BL1631 was on average 4.3-fold lower than on glucose (95% confidence interval between 3.4 and 5.6). This result together with the observed pattern in the microarray led us to conclude that the expression of BL1631 is regulated in a sugar-dependent manner and that the transcription is down-regulated in cells growing in the presence of lactose compared to glucose-grown cells.

**BL1631 encodes a glucose-specific permease, GlcP.** BL1631 was originally annotated as a xylose transporter, *xylT*, based on the highest BLAST similarity with the well-characterized xylose transporter XylT from *Lactobacillus brevis* (46% amino acid identity; accession number AAC95127) (4, 25, 26). Further analysis using the BLAST search at the TCDB, in which experimentally investigated targets are considered, revealed

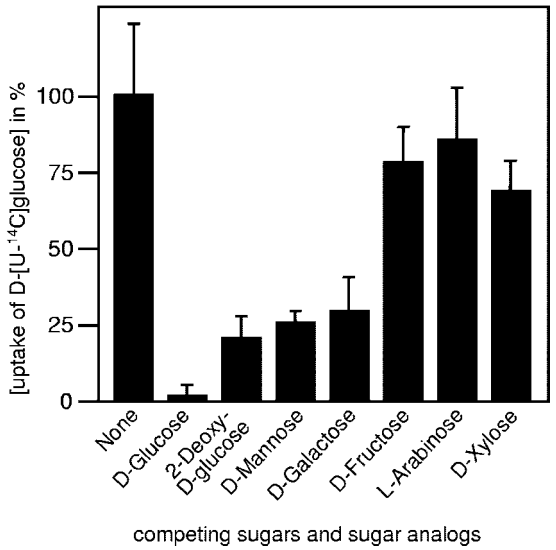


FIG. 3. Competition of sugars with glucose uptake by *E. coli* LM1(pMB1). The cells were grown in Luria-Bertani broth to $OD_{600}$ of 0.5. After one hour of further growth in the presence of 1 mM IPTG, glucose uptake was determined in the presence of 200 μM [$^{14}$C]glucose and 10 mM competitor sugar. The initial rate of glucose transport in *E. coli* LM1(pMB1) was 2 nmol $min^{-1}$ $OD^{-1}$ (100%). Glucose uptake activities are presented as a percentage of the uptake by cells grown on glucose.

significant identities to the glucose permeases GlcP from *Synechocystis* sp. strain PCC6803 (37% identity; accession number CAA34119) and *Streptomyces coelicolor* (33% identity; accession number CAC01642) (4, 25, 26). Based on the TCDB BLAST result together with the BL1631 gene expression profile and glucose transport results, we hypothesized that this gene encodes a glucose permease rather than a xylose transporter. To confirm this, we tested whether BL1631 was able to functionally complement *E. coli* strain LM1 that carries mutations in all intrinsic glucose transporter genes (15). LM1 was transformed with pMB1, in which the BL1631 coding region was cloned under control of the $P_{tac}$ promoter in pBluescript KS(−). LM1(pMB1) formed red colonies on MacConkey agar plates supplemented with 50 mM glucose and 1 mM IPTG due to fermentation of glucose, while the isogenic control LM1[pBluescript KS(−)] remained white. Subsequently performed glucose uptake experiments revealed that LM1(pMB1) effectively transported glucose at a rate of 2.6 nmol of glucose $OD_{600}^{-1}$ $min^{-1}$, while glucose uptake of LM1[pBluescript KS(−)] was negligible (<0.1 nmol of glucose $OD_{600}^{-1}$ $min^{-1}$).

To further characterize the properties of BL1631 in vivo, we analyzed its substrate specificity by measuring glucose transport in the presence of an excess of competing sugars. As can be inferred from Fig. 3, BL1631 showed the highest specificity for glucose, followed by 2-deoxy-D-glucose, mannose, and galactose. In contrast, fructose, arabinose, and xylose were poorly recognized. The $K_m$ and $V_{max}$ values for glucose uptake were determined to be 70 (±14) μM and 11 (±2) nmol $OD_{600}^{-1}$ $min^{-1}$ (not shown). Thus, BL1631 encodes a specific glucose permease and *xylT* was renamed *glcP*.

**Genetic organization of the *glcP* locus.** The genetic organization of the *glcP* locus is presented in Fig. 4. A gene (*pgm*) encoding a putative phosphoglucomutase of 558 amino acids and 57% protein identity to Pgm of *S. coelicolor* (SCO7443) is located 85 nucleotides downstream of *glcP*, followed by a potential transcriptional terminator. A second putative glucose transporter gene, *ptsG*, is located 499 nucleotides upstream of *glcP*, and is oriented in the opposite direction of *glcP*. It is predicted to encode an enzyme II permease of the glucose-glucoside phosphotransferase system family (21). With 34% identical residues, the product shares the highest similarity to the glucose-specific EII from *Corynebacterium glutamicum* ATCC 13032 (accession number L18875) (17). The *glcP-ptsG*

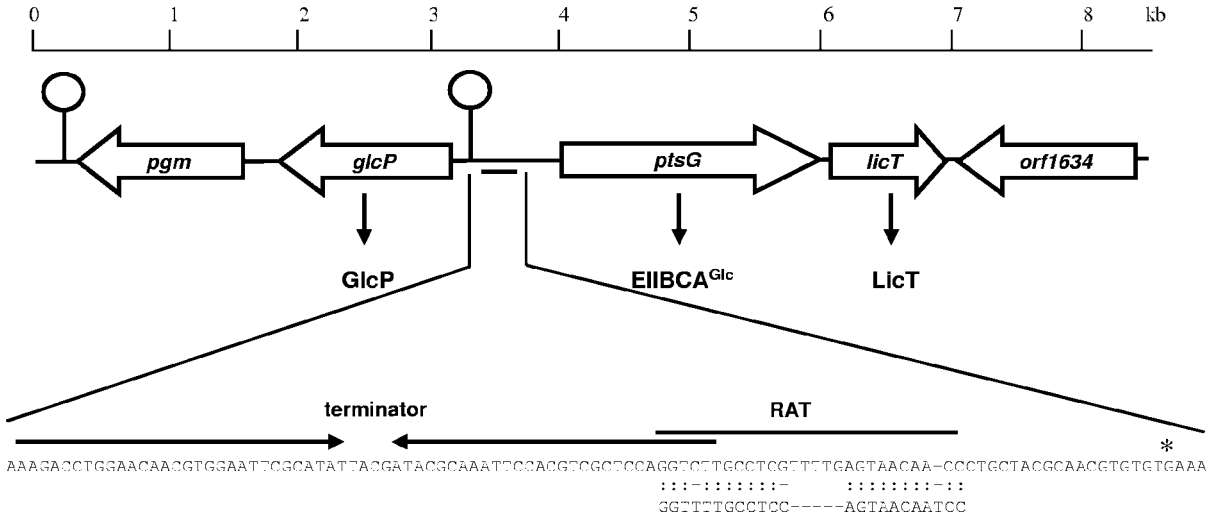


FIG. 4. The genomic organization of the *glcP* region in *B. longum*. The sizes and orientations of the genes were deduced from the nucleotide sequences. A part of the nucleotide sequence of the *glcP-ptsG* intergenic region is presented below the genetic organization, showing inverted repeats of the transcriptional terminator and of the RAT sequence, which is aligned with a RAT consensus sequence. A potential transcriptional terminator is indicated by inverted arrows and a stem-loop. A putative transcriptional start site of *glcP* determined by primer extension is indicated by an asterisk.

intergenic region comprises a potential rho-independent terminator and a ribonucleic antiterminator (RAT) element that overlap by six nucleotides (20). The RAT-terminator sequence might be the regulatory site of an antiterminator protein. A potential antiterminator, sharing 32% identity with *Bacillus subtilis* LicT (accession number CAA82194), is encoded by a gene located downstream of *ptsG*.

**Expression of other relevant genes.** Expression of the *glcP* locus genes, *pgm*, *ptsG*, and *licT*, examined by microarrays and real-time PCR showed no significant changes in response to glucose, lactose, or glucose and lactose. Expression of *ptsG* was barely detectable using microarrays and real-time PCR, suggesting an inferior role of *ptsG* in glucose transport in *B. longum* and *glcP* as the main target of lactose repression. No additional obvious glucose transporter-like genes were detected, neither in the genome of *B. longum* by sequence similarity nor by analysis of glucose-inducible genes obtained by microarrays.

In addition to *ptsG*, which is the single PTS transporter identified in the genome of *B. longum* NCC2705, the expression of general PTS genes *ptsI* and *ptsH* was also barely detectable in microarrays. The presence of only one PTS transporter and a weak expression of all PTS genes suggest the minor role of this system in the sugar metabolism of *B. longum*.

The genome of *B. longum* (accession number AE014295) contains four putative lactose operons. Two operons are identical, consisting of LacI-like regulator, β-galactosidase, and ABC transporter genes (BL0258-BL0260 and BL1167-BL1169). The third operon consists of genes encoding putative lactose permease (BL0976) and β-galactosidase (BL0978). The fourth, probably nonfunctional operon consists of a LacI regulator-like gene (BL1774) and a cryptic β-galactosidase-like gene (BL1775). The microarray data indicate that these genes are either constitutively expressed or induced by lactose, but none of them is significantly repressed by glucose (data not shown).

**Primer extension analysis of *glcP*.** A potential transcriptional start site of *glcP* was resolved by primer extension analysis in a carbon source-dependent manner. The result of the experiment depicted in Fig. 5 revealed that primer extension signals match with a G residue located 205 bases upstream of *glcP* and 19 bases upstream of a putative RAT sequence. Signals were stronger in RNA preparations from glucose-grown cells, compared to cells grown on lactose or both sugars, which is consistent with the observed lactose-mediated repression of *glcP* in real-time PCR and microarrays.

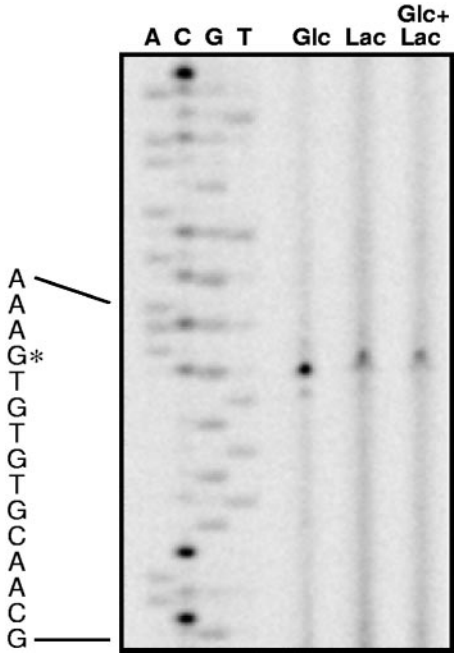


FIG. 5. Primer extension analysis of *glcP* gene transcription of *B. longum*. Total RNA was isolated from cells grown on SSM supplemented with 10 mM glucose (Glc), 5 mM lactose (Lac), and 10 mM glucose plus 5 mM lactose (Glc+Lac). Primer extension products, obtained with 15 μg of RNA, were separated along with sequencing reactions on an 8% polyacrylamide-urea gel. Products were analyzed on 8% polyacrylamide-urea gels. The determined transcriptional start site is indicated with an asterisk.

## DISCUSSION

In this study we demonstrated by a thorough analysis of sugar utilization that the strain *B. longum* NCC2705 preferentially uses lactose over glucose as a carbon and energy source. Such a "reversed diauxie" has been reported only for the low G+C gram-positive bacterium *Streptococcus thermophilus* (24). However, underlying mechanisms involved in this regulation seem to be different. *S. thermophilus* is highly adapted to growth on lactose but is, unlike *B. longum*, a poor fermenter of glucose. Our *B. longum* strain grows equally well on both sugars. A similar result was found in *B. longum* SH2 (11), which shows only a slightly higher growth rate on lactose than on glucose. Furthermore, in *S. thermophilus*, lactose is taken up via the highly efficient transport protein LacS in symport with a proton or antiport with galactose whereby galactose accumulates in the culture medium (6). Using [$^{13}$C]glucose in combination with nonlabeled [$^{12}$C]lactose, we showed that neither glucose nor galactose moieties of lactose were detectable in the culture medium, suggesting that both lactose moieties are rapidly metabolized by *B. longum*. Thus, the lactose-to-glucose preference in *S. thermophilus* is a consequence of the low efficiency of glucose transport and high efficiency of lactose transport. However, the lactose-over-glucose preference in *B. longum* seems to be regulated in a different way.

Glucose transport assays with *B. longum* revealed the downregulation of glucose transport by lactose. A genome-wide screening led to the identification of one target gene (BL1631) of lactose-mediated repression. Initially, bioinformatic analysis of BL1631 suggested a function as xylose transporter since it is clearly most similar to XylT of *L. brevis*, which exhibits the highest substrate specificity for xylose (4). However, gene expression results, complementation of a glucose-deficient *E. coli* strain, and data derived from substrate specificity analysis clearly support that BL1631 encodes a glucose-specific permease of the major facilitator superfamily, which likely functions as a glucose:$H^+$ symporter (18, 25) and therefore was named *glcP*.

Independent transcriptional analyses using microarrays, real-time PCR, and primer extension revealed lower levels of *glcP* expression in lactose-grown cells. The regulation of gene expression by different carbon sources usually involves a reg-

ulatory mechanism of carbon catabolite repression. This regulatory mechanism is not known to occur in *B. longum* NCC2705, especially since the genome contains no obvious catabolite repression elements such as a *ccpA*-like homologue, respective *cis*-acting catabolite responsive elements (*cre*), or HPr-kinase.

A mechanism of *glcP* regulation in *B. longum* might implicate the putative LicT-like antiterminator encoded two genes upstream from *glcP* (Fig. 4). Antiterminator proteins bind to RAT sequences to prevent the formation of an overlapping RNA terminator structure, thereby facilitating gene transcription (20). A RAT sequence was identified 19 nucleotides downstream of the *glcP* transcriptional start site partially overlapping with a predicted terminator structure (Fig. 4). Activation of antiterminator proteins usually occurs via phosphorylation of conserved histidine residues by the general PTS components in response to the availability of the substrate (16). Hence, one might speculate that *B. longum* LicT is activated in the presence of glucose upon depletion of lactose from the culture medium. Further analyses should be performed to prove a possible implication of LicT in antitermination of *glcP* and its activation by the products of poorly expressed PTS genes.

The *glcP-ptsG* locus described here as being involved in glucose uptake is opposed by three putative lactose operons in the genome of *B. longum*. Interestingly, the expression of these putative lactose operons seems not to be down-regulated in the presence of glucose, and β-galactosidase activity remained constitutive. These observations illustrate how well *B. longum* has adapted to growth on lactose. The potential to use lactose probably contributes to the adaptation to the gastrointestinal tracts of mammals in the first months of life, when lactose is a predominant carbon source.

In summary, the lactose-over-glucose preference in *B. longum* NCC2705 is manifested by lactose repression of the glucose transport. Data presented here suggest that this is (partially) achieved by down-regulation of *glcP* expression in a lactose-dependent manner, thereby shifting the balance of uptake and metabolism between glucose and lactose. More work is required to study the molecular mechanisms of lactose repression in *B. longum*. This is still hampered because development of cloning vectors and strategies to obtain mutants in bifidobacteria has not yet progressed sufficiently. The present work provides the basis to further analyze the phenomenon of lactose preference at the molecular level in bifidobacteria.

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